IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of

BIRCH, J. et al. Atty. Ref.: 4145-14

Serial No. 10/501,777 TC/A.U.: 1633

Filed: July 19, 2004 Examiner: Burkhart

For: GLUTAMINE-AUXOTHROPHIC HUMAN CELLS CAPABLE OF PRODUCING PROTEINS AND CAPABLE OF GROWING IN A GLUTAMINE-FREE MEDIUM

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December 1, 2010

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY BRIEF

Sir:

Appellant provides herein a response to aspects of the Examiner's Answer mailed October 4, 2010 for consideration by the Board in addition to the appellant's Appeal Brief filed July 12, 2010 and pursuant to 37 CFR § 41.41 and MPEP § 1208[1].

[1] The reply brief should include the following items, with each item starting on a separate page, so as to follow the other requirements of a brief as set forth in 37 CFR 41.37(c):
(A) Identification page setting forth the appellant's name(s), the application number, the filing date of the application, the title of the invention, the name of the examiner, the art unit of the examiner and the title of the paper (i.e., Reply Brief);
(B) Status of claims page(s);
(C) Grounds of rejection to be reviewed on appeal page(s); and
(D) Argument page(s).

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(1) STATUS OF THE CLAIMS

Claims 32-41 are pending.

The application has been twice rejected.

The originally-filed claims 1-9 were amended, without prejudice, in a Preliminary Amendment filed July 19, 2004.

Claims 2 and 9 were canceled, claims 10-15 added, and claim 1 amended, without prejudice, in an Amendment filed February 21, 2006.

Claims 6, 12, 13, 14 and 15 were amended and claims 16-24 added, without prejudice, in a Supplemental Amendment filed March 26, 2006.

Claim 1 was amended, without prejudice, in an Amendment After Final Rejection filed August 25, 2006. The Amendment After Final Rejection filed August 25, 2006 was entered with the filing of a Request for Continued Examination (RCE) on September 26, 2006.

Claims 1 and 7 were amended, claims 8, 12, 13 and 16 were canceled and claims 25-27 were added, without prejudice, in a Response filed April 30, 2007.

Claims 17, 21, 23 and 27 were amended, claims 25 and 26 were canceled and claims 28-31 were added, without prejudice, in an Amendment After Final Rejection filed October 25, 2007. The Amendment After Final Rejection filed October 25, 2007 was entered with the filing of a Request for Continued Examination (RCE) on October 25, 2007.

Claims 1, 10, 14, 17, 18, 21, 22, 23, 28 and 29 were amended, claims 11, 30 and 31 were canceled, and claims 32-35 were added, without prejudice, in an Amendment filed July 16, 2008.

Claims 1, 3-7, 10, 14, 15, 17-24 and 27-29 were canceled, without prejudice, in an Amendment After Final Rejection filed July 2, 2009. The Amendment After Final Rejection filed July 2, 2009 was entered with the filing of a Request for Continued Examination (RCE) on July 15, 2009.

Claims 32, 33, 34 and 35 were amended, without prejudice, and claims 36-41 added, in a Supplemental Amendment filed August 17, 2009. The Supplemental Amendment filed August 17, 2009 has been entered. See Interview Summary October 5, 2009 and Examiner Interview Summary dated October 13, 2009.

A copy of all the rejected claims 32-41, i.e., the claims involved in the appeal, is attached as a Claims Appendix to the appellant's Appeal Brief, pursuant to Rule 41.37(c)(1)(viii).

(2) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following ground of rejection are presented for review:

Whether the invention of claims 32-41 would have been obvious from the combined teachings of Wilson (WO 87/04462) or Bebbington (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821), in view of Brandt (U.S. Patent No. 6,395,484), Schneider (Journal of Biotechnology, Vol. 46, pages 161-185 (1996)), Gawlitzek (Biotechnology & Bioengineering, Vol. 68, No. 6, June 2000, pages 637-646) and Hermentin (U.S. Patent No. 6,096,555), as defined by 35 U.S.C. § 103.

(3) ARGUMENT

The methods of claims 32-41 would not have been obvious from the combined teachings of Wilson (WO 87/04462) or Bebbington (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821), in view of Brandt (U.S. Patent No. 6,395,484), Schneider (Journal of Biotechnology, Vol. 46, pages 161-185 (1996)), Gawlitzek (Biotechnology & Bioengineering, Vol. 68, No. 6, June 2000, pages 637-646) and Hermentin (U.S. Patent No. 6,096,555), and the rejection of claims 32-41 under 35 U.S.C. § 103 over the cited combination of art should be reversed. Consideration of the following, in addition to the remarks and arguments in the Appeal Brief filed July 12, 2010, in this regard is requested.

The Examiner's Answer asserts that in detailing the teachings of Gawlitzek,

> "applicants grasp a sentence fragment (page 641, first column) and insist this refutes all of the prior findings along with those of Gawlitzek et al. Read in light of the entire reference, this sentence fragment is taken out of context by applicants, as sentence fragments often are." See page 14 of the Examiner's Answer.

Page 14 of the appellant's Appeal Brief referred to page 641 of Gawlitzek as follows (emphasis added):

> "While Gawlitzek may mention in the introduction (last sentence, page 637 – with reference to Schneider et a. J Biotechnol. 1996), that numerous studies reported negative effects of elevated ammonium concentrations on cell growth and productivity of different cell lines, Gawlitzek is understood to have observed "no detrimental

> effect of high ammonium on cell growth or viability" and "cell-specific productivity of TNFR-IgG was similar in all cases" (page 641, first paragraph, left column; Figure 1). In other words, Gawlitzek was unable to confirm the findings described by others such as Schneider et al. Gawlitzek neither teaches nor suggests a method to extend cell viability or a method to increase sialylation by way of transfecting a cell line with a GS gene, as claimed."

The following is a reproduction of the entire paragraph spanning pages 640-641 of Gawlitzek from which the quoted passage was obtained (emphasis added);

> **Effect of Ammonium on Carbohydrate Composition of TNFR-IgG**
> CHO cells producing TNFR-IgG were cultivated in the presence of various ammonium concentrations achieved by adjusting glutamine and NH_4Cl levels. Primary 10-L bioreactors were used to grow cells to the desired cell concentration before inoculating duplicate 3-L bioreactors for each ammonium level studied (see Materials and Methods and Fig. 1). Ammonium concentrations were measured throughout the cell culture time course (Fig. 2). In the growth phase of the cultures, the ammonium concentration increased from a starting level of ~0.9 m*M* to ~6.5 m*M* on day 4 (data not shown). Ammonium levels remained fairly stable and reflected the medium used after cells were transferred into 3-L bioreactors on day 4. By using the experimental setup, we could examine the effect of a range of biotechnologically relevant ammonium concentrations (~1 to 15 m*M*) on the N-glycans of TNFR-IgG. No detrimental effect of high ammonium levels on cell growth or viability was observed. Cell-specific productivity of TNFR-IgG was similar in all aspects. The cell culture process was terminated on day 10 and secreted TNFR-IgG was purified by protein-A chromatography for N-glycan analysis."

As demonstrated above, Gawlitzek concluded, in complete sentences, that "No detrimental effect of high ammonium levels on cell growth or viability was observed. Cell-specific productivity of TNFR-IgG was similar in all aspects." Moreover, these conclusions were based on experiments designed by Gawlitzek to "examine the effect of a range of biotechnologically relevant ammonium concentrations (~1 to 15 m*M*) on the N-glycans of TNFR-IgG."

This conclusion of Gawlitzek is contrary to the review by Schneider, which states that

> "Ammonium has been reported to be toxic and inhibitory for mammalian cell cultures for many years. Reduction of growth rates and maximal cell densities in batch cultures, changes in metabolic rates, perturbation of protein processing and virus replication have been reported. However, cellular mechanisms of ammonia toxicity are still the subject of controversy and are presented here. ... Strategies to overcome toxic ammonium accumulation include substitution of glutamine for glutamate or other amino acids, nutrient control, i.e., controlled addition of glutamine at low concentrations, or removal of ammonia or ammonium from the culture medium by means of ion-exchange resins, ion-exchange membranes, gas permeable membranes or electrodialysis." See Abstract of Schneider.

As stated in the appellant's Appeal Brief, Gawlitzek was unable to confirm the findings described by others such as Schneider.

The Examiner states on page 14 of the Examiner's Answer that the Gawlitzek conclusions that "No detrimental effect of high ammonium levels on cell growth or viability was observed," and "Cell-specific productivity of TNFR-IgG was similar in all

aspects." were the result of experimental design relating to a shift of temperature by Gawlitzek during fermentation from 37°C to 31°C "to delay the onset of apoptotic cell death."[2]

The Examiner's assertion and "alternative explanation" in this regard is unsupported by any evidence of record and, to be accepted, would apparently require a conclusion that Gawlitzek was imprecise and incorrect in describing the above-quoted "RESULTS" section as demonstrating the "**Effect of Ammonium on Carbohydrate Composition of TNFR-IgG**".

The Appeal Brief further stated that

> "In fact, the Discussion of Gawlitzek taken with the results of Figure 7 of Gawlitzek, for example, indicate that limiting or eliminating glutamine in the model CHO cell of Gawlitzek will not effect terminal galactosylation and sialylation." See page 14 of the Appeal Brief.

The Examiner's Answer at page 14 criticizes this conclusion.

The appellants again note however that Gawlitzek reports in Figures 6 and 7 of the article the results of an experiment whereby increased ammonia concentrations in the absence of glutamine did not effect the mRNA levels or enzyme activities of β1,4-Galactosyltransferase and α2,3-sialyltransferase (i.e., enzymes responsible for galactosylation and sialylation) as compared to controls with no

[2] "The results applicants are referring to in this instance, i.e., Figs. 1 and 2, include cells that were shifted to a temperature of 31°C in order to prevent apoptosis (or cell death), see the ¶ linking the first

ammonia in the absence of glutamine. The "**Bioreactor Experiments**" section of the "**MATERIALS AND METHODS**" of Gawlitzek describes these experiments as having been performed at a constant pH.[3] The finding of no direct effect of ammonia in these experiments is consistent with the conclusion of Gawlitzek that

> "The results presented here strongly suggest that ammonium inhibits galactosylation and sialylation of TNFR-IgG N-glycans by pH-regulated mechanisms. We hypothesize that ammonium decreases α2,3-sialyltransferase and β1,4-galactosyltransferase activities by increasing the pH of the *trans*-Golgi compartment." See page 644, right column, first two sentences of the last paragraph, of Gawlitzek.

Gawlitzek suggests therefore that regulation of pH in the model CHO cell of Gawlitzek could overcome ammonium related decreases α2,3-sialyltransferase and β1,4-galactosyltransferase activities. The appellants believe it is reasonable to conclude from Gawlitzek that limiting or eliminating glutamine in the model CHO cell of Gawlitzek will not effect terminal galactosylation and sialylation

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and second columns, page 638. This is an alternative explanation regarding the cell viability results by applicants." See page 14 of the Examiner's Answer.

[3] " Reactors were equipped with calibrated dissolved oxygen, pH, and temperature probes. Dissolved oxygen was controlled on-line through sparging with air and/or oxygen at 60 ± 5% air saturation, pH was maintained through on-line addition of CO_2 or Na_2CO_3 at pH 7.2 ± 0.1." See page 638, right column of Gawlitzek.

Page 12 of the Examiner's Answer includes new arguments relating to the claimed requirement that the viability of the cell of the claims is extended as a result of the claimed method.[4]

The Examiner specifically asserts that

> "In a general sense, the instant specification only discloses extending cell viability using media supplements (page 10, lines 15-25), not by use of the glutamine synthetase transgene. In a narrow sense, a single clone, 3E 10, was found to have extended viability in Example 10."

The relevance of the Examiner's attempt to distinguish between a general and narrow "sense" of the specification is not understood. The specification provides an adequate written description of the claimed invention and the specification teaches one of ordinary skill how to make and use the claimed invention. The claims are

[4] "Claim 32 recites in the preamble that the method is, inter alia, for "extending the viability of said cell", and a similar limitation is recited in the last line of the claim. In a general sense, the instant specification only discloses extending cell viability using media supplements (page 10, lines 15-25), not by use of the glutamine synthetase transgene. In a narrow sense, a single clone, 3E 10, was found to have extended viability in Example 10. Although not specifically stated, this is implied to be due to the glutamine synthetase transgene. Claim 32 is not limited to any specific cell type or clone, thus, the instant specification relies as much upon the prior art for this limitation as does the above rejection because of the lack of any broad, general teaching that the addition of a glutamine synthetase transgene will provide the claimed increase in cell viability. The general concept and thrust of the invention as disclosed is towards the use of the glutamine synthetase transgene in transfected cells in order to mediate glycosylation of proteins, not towards the extension of cell viability. Furthermore, cell viability is extended in the instant claims as compared to what? The parent cell line, untransfected with glutamine synthetase? Any chosen cell line or cell type? Given the above and the teachings of the prior art regarding the benefits of using glutamine-free media and cells competent to grow in such media, it is considered that this limitation is met by the combination of the prior art references above. In particular, the teachings of Schneider et al regarding the inhibitory nature of ammonia (a by-product of glutamine metabolism) in cell culture necessarily leads to a conclusion that reducing ammonia levels, e.g. by removing glutamine from the cell culture, would relieve cells from this inhibition, thus extending viability."

definite. There is no issue of written description, enablement or definiteness which is the subject of the present appeal.

The results and disclosure of Example 10 of the specification, for example, supports the claimed requirement of extended viability.

The Examiner's conclusion that

> "the teachings of Schneider et al regarding the inhibitory nature of ammonia (a by-product of glutamine metabolism) in cell culture necessarily leads to a conclusion that reducing ammonia levels, e.g. by removing glutamine from the cell culture, would relieve cells from this inhibition, thus extending viability." (emphasis added)

is not supported by the conclusions of Schneider. Specifically, Schneider does not "necessarily lead" to a conclusion that reducing ammonia levels "by removing glutamine from the cell culture" would overcome the perceived toxic effects of ammonia. Rather, Schneider concludes, among other things, that

> "Ammonia removal systems allow reduction in ammonia inhibition of mammalian cell cultures. Such systems include gas permeable, hydrophobic porous membranes, ion exchange membranes or ion-exchange resins and electrodialysis. ...
>
> While a considerable amount of literature has appeared during the last 30 years, progress in mammalian cell technology appears to be limited. This possibly reflects the fact that, despite all the efforts made, the underlying principles of regulation and utilization of different metabolites by mammalian cells remain poorly understood." See page 180, right column of Schneider.

Accordingly, Schneider expresses a considerable amount of uncertainty in the art, as opposed to the Examiner's characterization of a necessary conclusion.

The presently claimed invention defines methods which provide unexpected benefits in increasing sialylation and/or N-glycan charge of a glycosylated protein and in allowing for a greater rate of protein synthesis and increased maximum product concentration. These results would not have been expected from the cited combination of art. The claimed invention requires, as a result of the claimed method, the increase in sialylation and/or N-glycan charge of the glycosylated protein and extension of the viability of the cell of the claim.

The cited combination of art fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an exogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge and extension of cell viability, as claimed.

For reasons including those set forth above and in the Appeal Brief filed July 12, 2010, it is respectfully requested that the Section 103 rejection be reversed and the pending claims be indicated as allowable.

The claims are submitted to be in condition for allowance and Reversal of the outstanding rejection is requested.

Respectfully submitted,

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